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Antigen-based immunotherapy (AIT) for autoimmune and allergic disease

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Autoimmune and allergic diseases are major causes of morbidity. Antigen-based immunotherapy (AIT) is immunologically the most satisfying means of specifically targeting only those T cells driving disease, thereby inducing antigen-specific immune tolerance, with the lowest adverse risk profile. AIT is highly effective in rodent models of T cell-driven inflammation and is now in clinical trials. The range of approaches to applying AIT in the clinic prevents a consensus on the molecular basis for this form of tolerance. In particular, there has been a paucity of information on how pre-activated effector and memory T cells respond to AIT. New, advanced murine models of AIT are beginning to deliver such information at the cellular, biochemical, transcriptional and epigenetic levels.

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Introduction

Autoimmune diseases and allergic conditions are caused by inappropriate immune responses that attack either self-tissues or innocuous environmental antigens, respectively. Most current treatments for these conditions target the symptoms of the diseases rather than offering cures. Many of these treatments, especially in the context of autoimmunity, lead to significant immunodeficiency that can expose the patient to severe, sometimes life-threatening, infections. Such disease-modifying drugs are usually given over a long period of time, often for the lifetime of the individual. By contrast, specifically removing or modifying only those immune cells that drive the allergic

or autoreactive immune response could lead to a permanent cessation of the disease — a cure.

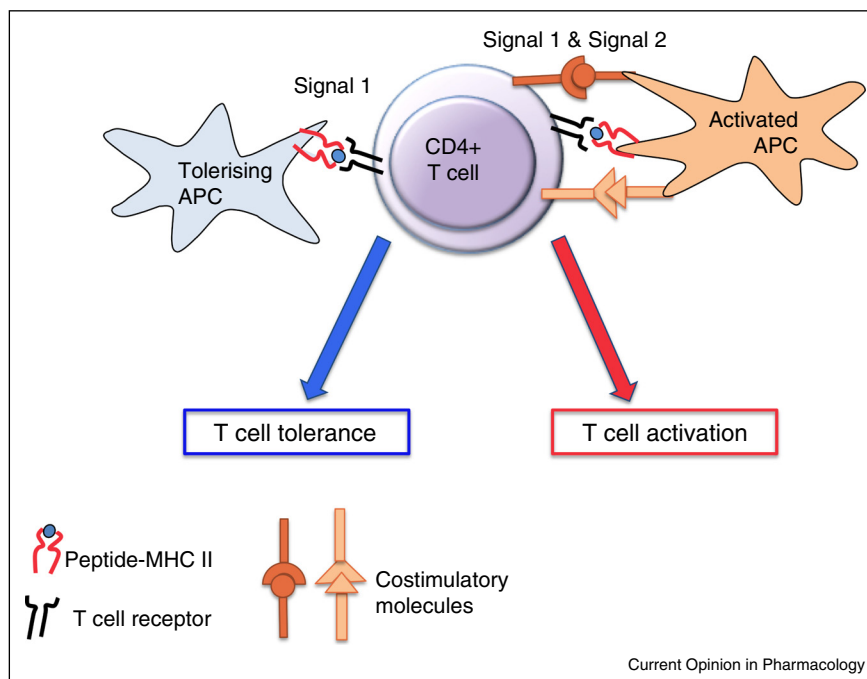
The CD4⁺ T cells believed to initiate and drive many autoimmune and allergic conditions are activated through their cell surface T cell receptor (TCR) that specifically recognises the self-antigen or allergen. By targeting the TCR, AIT aims to silence the damaging T cells whilst leaving the rest of the immune system intact. Success in animal studies and historical allergy treatments in humans has led to clinical studies in both fields. Here we will consider recent examples of AIT in both animal and human studies and discuss the underlying immunological mechanisms.

Immune tolerance — a physiologic basis for immunotherapy

T cells gain their heterodimeric TCR through random gene re-arrangement during thymic development. This is essential to generate a broad repertoire of T cells that can respond to the variable array of pathogens the host will be exposed to over a lifetime. TCR gene re-arrangement also generates T cells with the potential to be activated by self-peptides. Many of these potentially destructive T cells are deleted during negative thymic selection [1]. This ‘central tolerance’ is not complete, however. In all individuals, some self-reactive T cells mature and migrate to the periphery where, if activated, they have the potential to cause autoimmunity. To prevent this, ‘peripheral tolerance’ mechanisms regulate T cell activation to both self and innocuous foreign antigens, such as the dietary and environmental antigens that can cause allergy.

A key mechanism by which peripheral tolerance is maintained is the presentation of antigen in non-inflammatory contexts. Full T cell activation requires two signals stemming from cell surface receptors delivered when the T cell interacts with an antigen presenting cell (APC). Signal 1 is provided by TCR binding to short antigenic peptides presented by MHC molecules. Signal 2 (costimulation) is delivered by a number of different receptor–ligand pairs, upregulated in inflammatory environments. Delivery of signal 1 alone leads to T cell tolerance either as a consequence of T cell death, modulation of the T cell's ability to respond to further activation (often called anergy), or the T cell gains regulatory function, capable of suppressing the activation of other immune cells (Figure 1) [2–4]. AIT aims to mimic these natural tolerogenic processes by delivery of the appropriate self- or allergen-derived peptide in the absence of inflammatory

Figure 1



T cell activation requires two signals. CD4⁺ T cells are tolerised if they encounter their antigen (signal 1) presented by an antigen presenting cell (APC) that has not been activated. Inflammation and/or infection cause APCs to upregulate costimulatory molecules that deliver signal 2 to T cells. T cells that receive both signals 1 and 2 become activated and differentiate into effector cells. In the context of infection, effector T cells co-ordinate the clearance of invading pathogens. If the T cells recognise allergen or self-antigens, the host can develop allergy or autoimmune disease respectively. If a T cell receives a tolerising TCR signal in advance of encountering its antigen presented by an activated APC, the T cell will not become fully activated — it is tolerant.

signals. This attractively simple concept belies a complex process which requires extensive knowledge of the target antigen; the location, phenotype and function of the 'disease-causing' T cells; and an ability to track these T cells to evaluate the success of any treatment.

Allergy

For over 100 years [5], immunotherapy has been practiced in allergic individuals. Initially involving crude allergen extracts, antigens are now carefully prepared to reduce adverse events. Allergen-derived peptides rather than whole antigens are often used as peptide immunotherapy (PIT), particularly as these can be selected not to cross link allergen-specific IgE, a process that can lead to enhanced rather than reduced allergic symptoms, including anaphylactic shock [6]. Increasing the dose of the allergen over a period of months to years is thought to modulate the allergen specific immune response including inducing a switch from an IgE dominated antibody response to IgG [7] and/or production of the regulatory cytokine interleukin (IL)-10 [8]. More recent advances in the ability to track allergen-specific T cells in the blood of patients undergoing immunotherapy suggest that AIT causes the deletion, rather than the modulation, of the disease causing T cells [9[•],10^{••}].

The route of delivery of the immunotherapy is likely to affect the dose of antigen that reaches the immune system. In Europe, the sublingual route is approved for allergy immunotherapy while the subcutaneous route is approved in the USA [5]. While the efficacies of these two routes have rarely been compared directly, a recent meta-analysis indicates that tablets designed to treat grass pollen allergy and subcutaneous delivery of pollen antigens can each reduce symptoms of rhinoconjunctivitis to a similar extent [11[•]]. Delivery of antigen can also be increased by either direct injection into lymph nodes with the aid of ultrasound or by binding the antigen to particular adjuvants such as aluminium hydroxide [7,12].

Autoimmune disease

In allergy, the inciting antigen is usually well-defined. This is far from the case for many autoimmune diseases. Considerable progress has been made in recent years in the identification of potential antigens in many autoimmune conditions [13–20]. However, pinning down the specificity of the T cells that cause these diseases is a complex process, further confused by the accumulation of different antigenic targets as the disease progresses, a process known as epitope spreading [21].

Despite these complexities, a number of human trials have taken place or are currently ongoing. Individuals considered at risk of diabetes have been given insulin or the pancreatic enzyme, glutamine decarboxylase, Gad-65, in an attempt to remove or modify the immune cells that cause pancreatic β -cell destruction (reviewed in [22]). These studies have found no delay in, or modification of, disease progression. Such trials, as Harrison *et al.* suggest, are unlikely to provide meaningful data without biomarkers to evaluate the impact of the therapy [22].

Similar approaches have been made to reduce disease progression in individuals with multiple sclerosis. Myelin peptides delivered alone, in the context of self-MHC molecules, or chemically fixed to patient cells have had a variety of effects from some reduction in relapses, no effect, or, in some cases, serious hypersensitivity (reviewed in [23]). Hypersensitivity responses were found in studies aiming to modulate the T cell response using peptides with a slightly altered sequence compared to the self peptide. Such altered peptide ligands (APLs) have different TCR-binding affinities and consequently provide altered signals for T cell activation leading to quantitative and/or qualitative changes in the T cell response. These effects are often specific for individual TCRs, which makes clinical translation from reductionist animal models to outbred human populations with more complex TCR repertoires inevitably unreliable.

Modulation of T cell responses has also been observed in rheumatoid arthritis (RA) patients given a peptide from dnaJ, a heat shock protein, which shares similarities with the RA 'shared epitope' [24,25]. Oral delivery of this peptide led to improved clinical responses and a shift from inflammatory to regulatory cytokine production. By contrast, intranasal delivery of a potential RA autoantigen, human cartilage glycoprotein-39, failed to provide any additional benefit compared to placebo [26]. More recent phase 1 studies in RA patients, and also in diabetic patients, have delivered the tolerising antigen using patient derived APCs. Dendritic cells (DCs), a key population of APCs, are differentiated from the patient's blood cells in the presence of tolerogenic signals, incubated with antigens before transfer back into the patient [27,28]. Such approaches ensure that the antigen is targeted to APCs that are likely to interact with the disease-causing T cells and can ameliorate disease in animal models by either reducing T cell effector responses and/or increasing immune regulation [29,30]. Likewise, antigen can be targeted to DCs by binding it to nanoparticles. Such approaches have been successfully used in animal models of allergy and autoimmunity [31,32].

As in allergy trials, the ability to track the target T cell population will be crucial to evaluate the success, and investigate the mechanisms of, disease modification. Recent studies using MHC class II tetramers containing

RA-associated peptides show that, while healthy controls and RA patients have similar numbers of RA-relevant T cells, the T cell populations from patients are more likely to have a memory T cell phenotype [33]. Memory T cells are generated following immune responses and may, as discussed below, present a more complex challenge to immunotherapy.

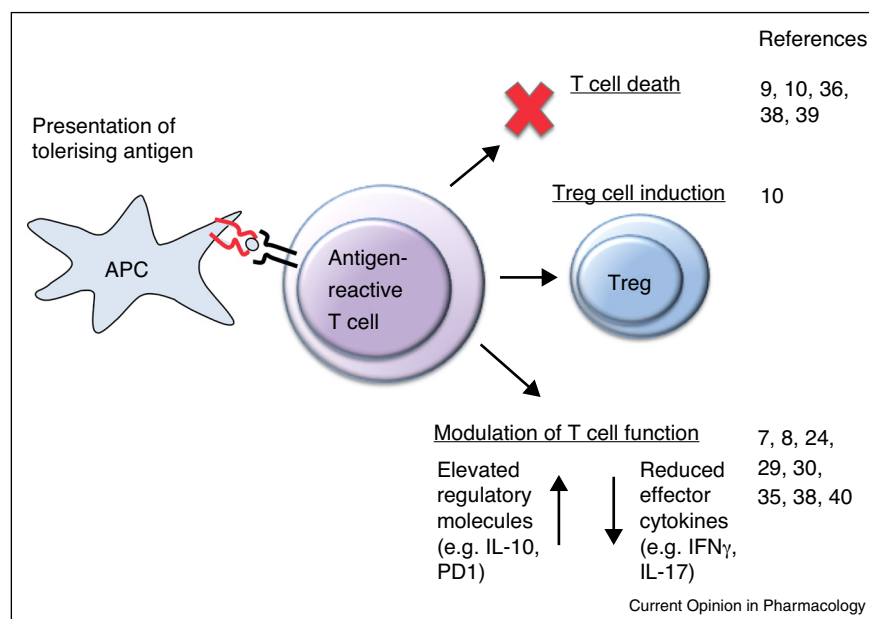
Mechanisms of action

Many animal studies have investigated the mechanisms of tolerance induction following AIT (Figure 2 and reviewed in [34]). Recent full genome transcriptional analysis of T cells exposed to a dose escalation of peptide suggest that, as in some of the human trials discussed above, AIT shifts T cells towards production of the regulatory cytokine, IL-10 [35]. In other experimental scenarios, a single high dose of AIT can drive T cells to apoptosis [36].

Many of these studies have, however, investigated the mechanisms of AIT in the context of naïve T cells. Allergen or autoreactive T cells either involved in ongoing or previous disease will be effector or memory T cells. These previously activated T cells show responses that are distinct from those of naïve T cells. Critically, they can be activated by lower levels of antigen and do not necessarily require costimulatory signals to make an effector response [37]. These differences suggest that effector and memory T cells will fail to be tolerised by AIT, instead potentially causing more immune-driven damage.

Our recent studies have investigated the consequences of exposing memory T cells to AIT. In a mouse allergy model, this response varied depending on the phenotype of the targeted memory cells with effector memory T cells undergoing a greater reduction in their subsequent responses than central memory T cells [38]. This might have consequences for when best to apply AIT in seasonal allergies. Effector memory T cells would be expected to be dominant during active allergen exposure, whereas immune memory during the 'off-season' might be maintained predominantly by central memory T cells. A good knowledge of the target cell population is, therefore, likely to be key to selecting the most effective treatment strategy. Similarly, while memory T cells proved to be initially resistant to AIT, they were altered such that further activation led to apoptosis [39]. These data suggest that memory cells retain information about their activation history which affects their subsequent responses. Potentially this cellular memory is retained via epigenetic changes to the cell's DNA. Indeed, we recently demonstrated that effector T cells silenced by AIT must express the cell surface protein, PD-1 [40]. Ligation of this inhibitory receptor serves to diminish TCR-driven T cell activation/function and its expression is usually tightly related to TCR ligation. AIT led to DNA

Figure 2



Mechanisms of action of AIT. AIT can alter the responses of allergen- or self-reactive T cells via a number of mechanisms. In some cases, the pathologic T cells are deleted following recognition of antigen on tolerising APCs. Alternatively, these T cells can be modulated either differentiating into regulatory T cells that can suppress the immune response, or altering their effector response by decreasing inflammatory cytokine production or increasing expression of immunomodulatory molecules such as IL-10 or PD-1.

demethylation within the PD-1 gene promoter, enabling long-term expression of the receptor. These data begin to provide a molecular basis for the loss of pathogenic T cell function following AIT.

Future perspectives

From its demonstrated effectiveness in rodent models of autoimmune and allergic diseases, AIT has reached implementation in clinical trials. Key to translational success will be rigorous interrogation of molecular mechanisms, particularly in T effector and T memory cells. The use of peptide-MHC tetramer staining can identify epitope-specific T cell in a range of human diseases, most notably allergies where the inciting antigen is well characterized. This remains more of a challenge for autoimmune diseases with complex autoaggressive T cell repertoires. A variety of treatment regimes — routes, doses, delivery systems and proteins versus peptides — are being trialled. Reaching a consensus from the likely different outcomes will be challenging at the molecular level. It is necessary to specifically focus on those T cells with TCRs recognizing the therapeutic antigen. Low cell yields from patients will make large scale, unbiased, 'omics' analyses impractical. For this we must rely on more advanced mouse models that allow retrieval of sufficient material (tolerant T cells) to identify alterations in key genes/pathways that then can be interrogated more easily in human studies. These might provide useful biomarkers (in concert with tetramer staining) for confirming the

establishment of tolerance and, importantly, for signs that it is beginning to wane, prompting renewed immunotherapy.

Conflict of interest statement

Nothing declared.

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